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*informal communication*

To Joyce Tung  
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From Leda Trivinos

Re

Applicant : Zervos et al.  
Serial No. : 09/245,549  
Filed : February 5, 1999

Art Unit : 1637  
Examiner : Joyce Tung

Title : IN VIVO CONSTRUCTION OF DNA LIBRARIES

Number of pages  
including this page

*5*

Message Dear Examiner Tung:

Attached is a set of proposed amendments to claims 1, 3 and 26 in the above application. Please review the amendments and contact me to discuss them.

These proposed amendments are for discussion purposes only- Please do not enter this amendment.

Regards,

*Leda Trivinos*

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Attorney Docket No.: 10284-019001

1. A method for constructing a DNA library in vivo, comprising:
  - providing a plurality of host cells;
  - providing a vector molecule having a first region and a second region;
  - providing a plurality of nucleic acid insert molecules, each of the plurality of nucleic acid insert molecules having a first common region which is homologous with said first region of the vector molecule, a second common region which is homologous with said second region of the vector molecule, and a library element encoding region disposed between said first common region and said second common region, wherein when the library element encoding region encodes a naturally occurring sequence, the first and second regions are not naturally found adjacent to the library element encoding region;
  - introducing the vector molecule into each host cell of the plurality of host cells;
  - introducing a nucleic acid insert molecule from said plurality of nucleic acid insert molecules into each host cell of said plurality of host cells, wherein a different library element encoding region is introduced into each host cell of said plurality of host cells;
  - allowing homologous recombination and gap repair between a vector molecule and a only one nucleic acid insert molecule to occur, and
  - generating a plurality of vector molecules from said plurality of host cells, each vector molecule of the plurality comprising a different ~~nucleic acid insert~~ library element encoding region, and each vector comprising only one library element encoding region.
- thereby constructing a DNA library.

2. Previously canceled

3. A method of constructing a DNA library, comprising:

- providing a plurality of nucleic acid molecules wherein each of said nucleic acid molecule includes, in order from 5' to 3', a first common sequence, a library element encoding region, and a second common sequence;
- providing a plurality of first primers, each of said first primers having a first region which hybridizes to the first common sequence of the nucleic acid molecule and having a second region which does not hybridize to said first or second common sequence;
- providing a plurality of second primers, each of said second primers having a first region which hybridizes to the second common sequence of the nucleic acid molecule and having a second region which does not hybridize to said second or first common sequence;
- forming a reaction mixture which includes said plurality of nucleic acid molecules, said plurality of said first primers, and said plurality of said second primers, under conditions which provide a plurality of nucleic acid insert molecules having the following structure, in order from 5' to 3', a second region of said first primer/said first common region/a library element encoding region/said second common region/a second region of said second primer;
- providing a plurality of host cells;
- providing a vector molecule having a first region which is homologous with said second region of said first primer, and a second region which is homologous with said second region of said second primer;
- introducing said vector molecule into each host cell of the plurality of host cells; and
- introducing one or more of nucleic acid insert molecules from said plurality of nucleic acid insert molecules into each host cell of the plurality of host cells;
- allowing homologous recombination and gap repair to occur between a vector molecule and only one nucleic acid insert molecule; and

generating a plurality of vector molecules from said plurality of host cells, each vector molecule of the plurality comprising a different nucleic acid insert molecule library element encoding region, and each vector comprising only one library element encoding region,

thereby providing a DNA library.

4. Cancel

26. A method of constructing a DNA library for screening in a two-hybrid system, comprising:

providing a plurality of nucleic acid molecules, wherein each of the plurality of nucleic acid molecules includes, in order from 5' to 3', a first common sequence, a library element encoding region, and a second common sequence;

providing a plurality of first primers, each of said first primers having a first region which hybridizes to said first common sequence of said nucleic acid molecule and having a second region which does not hybridize to said first or second common sequence;

providing a plurality of second primers, each of said second primers having a first region which hybridizes to said second common sequence of said nucleic acid molecule and having a second region which does not hybridize to said second or first common sequence;

forming a reaction mixture which includes the plurality of nucleic acid molecules, the plurality of said first primers, and the plurality of said second primers, under conditions which provide a plurality of nucleic acid insert molecules having the following structure, in order from 5' to 3', a second region of the first primer/the first common region/a library element encoding region/the second common region/a second region of the second primer;

providing a plurality of host cells;

providing a vector having a first region which is homologous with the second region of the first primer, and a second region which is homologous with

the second region of the second primer, wherein said vector further includes a transcription factor activation domain;

introducing a the vector molecule into each ~~of each~~ host cell of said plurality of host cells;

introducing one or more of the nucleic acid insert molecules into each host cell of said plurality of host cells under conditions which allow for recombination and gap repair to occur;

allowing homologous recombination and gap repair to occur between a vector molecule and only one nucleic acid insert molecule in each host cell of the plurality of host cells, wherein each host cell of the plurality comprises a vector molecule that comprises (a) a different nucleic acid insert molecule library element encoding region, and (b) only one library element encoding region,

introducing into each host cell of said plurality of host cells a nucleic acid molecule encoding a hybrid protein, wherein the hybrid protein includes a transcription factor DNA-binding domain attached to a test protein;

introducing into each host cell of said plurality of host cells a detectable gene, wherein said detectable gene comprises a regulator site recognized by the DNA-binding domain and wherein said detectable gene expresses a detectable protein when the test protein interacts with a protein encoded by the DNA library;

plating each host cell of said plurality of host cells onto selective media;

and

selecting for each host cell of said plurality of host cells containing a DNA encoded protein which interacts with test protein.

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